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Adding benefit to wetlands – Valorization of harvested common reed through mushroom production

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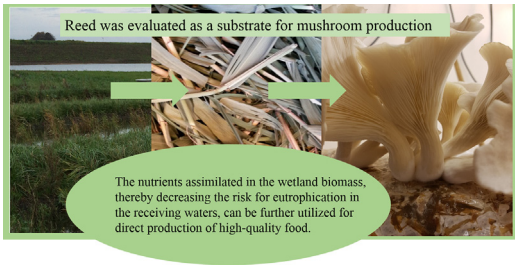
HIGHLIGHTS

- Reed was harvested from a constructed wetland in southern Sweden.
- Reed biomass was used as a substrate for production of oyster mushrooms.
- The biomass supported high production of high-quality fruiting bodies.
- Nutrients assimilated in reed biomass can be used for direct production of food.

GRAPHICAL ABSTRACT



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ABSTRACT

Wetlands have been successfully implemented as water purification systems for removal of plant nutrients and can play a significant role in nutrient recycling, depending on use of the harvested biomass. In a constructed wetland in southern Sweden examined in this study, assimilation of plant nutrients in wetland biomass corresponded to 234 kg/ha nitrogen, 22.8 kg/ha phosphorus, and 158 kg/ha potassium in the study year (2016). The harvested biomass, composed exclusively of common reed, was evaluated as a substrate for production of oyster mushrooms, one of the most widely produced edible mushrooms in the world. The biological efficiency of the substrate was 138 ± 10%, corresponding to production of 1.4 kg mushrooms (fresh weight) based on 1 kg reed (dry weight). The fruiting bodies had high quality, with total protein concentration 18.3 ± 2.8% and very low levels of contaminating heavy metals. Thus, nutrient assimilation in wetland biomass not only decreases the risk of eutrophication in recipient waters, but can be utilized for direct production of high-quality food. The biomass remaining after mushroom production, composed of mycelium and partly degraded wetland biomass, has potential for use in ruminant feed, i.e., as roughage.

1. Introduction

For more than three decades, constructed wetlands have been successfully used as water purification systems for removal of nitrogen and phosphorus from several types of nutrient-rich water, such as run-off from agricultural land and domestic wastewater (Nichols, 1983; Verhoeven et al., 2006; Kadlec and Wallace, 2009; Mitsch and Gosselink, 2015). While the main desired ecosystem function of constructed wetlands is to reduce plant nutrient losses to receiving water bodies (rivers, lakes, seas), several additional benefits can be derived from these ecosystems. These benefits include improved biodiversity in agricultural landscapes (Hansson et al., 2005; Thiere et al., 2009) and wetland-based recreation (Liquete et al., 2016). In addition, wetlands can play a significant role in recycling nutrients through

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macrophyte nutrient uptake, followed by biomass harvesting (Brix, 1997; Okurut, 2001; Kyambadde et al., 2004; Kadlec and Wallace, 2009). Harvested wetland macrophytes have several potential applications, e.g. as feed for farm animals or as a construction material for roofing (Terer et al., 2012; Köbbing et al., 2013).

In parallel with water purification requirements, the growing world population demands increased food production and there is a need for new food production systems that are well-suited to a bio-based society (Specht et al., 2013). This calls for a broader understanding and innovative ways to recycle nutrients from otherwise unused biomass resources back into food production. Harvested biomass from wetlands can be of interest in this regard.

Edible white-rot fungi, such as oyster mushrooms, can be grown on lignocellulosic plant material provided it has a suitable carbon/nitrogen ratio and a physical structure that allows gas exchange during fungal growth (Stamets, 2000). Oyster mushrooms are well-known as an aggressive colonizer of a wide array of substrates and are one of the most widely produced mushroom species in the world (Fernandes et al., 2015).

In Sweden, the market is currently dominated by white button mushrooms, but there is increasing interest among consumers in exotic species, such as oyster mushrooms (Richardsson, 2014). Besides adding new flavors and textures to the food, mushrooms are considered an important future protein source, with protein levels of 20–25% of dry weight (Kalac, 2013). Thus, increased production of mushrooms has great potential, contributing to increased sustainability in food production and allowing nutrient recirculation and production of an alternative protein source. Development of mushroom substrates based on unused biomass such as wetland plants can be an effective measure to reduce plant nutrient losses in runoff water from agricultural land.

The present study focused on biomass of common reed from constructed wetlands in southern Sweden. The content of plant nutrients in the harvested biomass and the potential for using it as a substrate for oyster mushroom production were studied. Furthermore, the biomass remaining after mushroom production, composed of mycelium and partly degraded wetland biomass, was analyzed for its potential as an animal feed, to fully exploit the potential for nutrient recycling. The presence of potential contaminants and the risk of transmission of these into the food chain must be considered in any residue-based process. Concentrations of cadmium and lead in the wetland biomass before and after (spent substrate) mushroom production and in the harvested mushrooms were therefore also studied.

2. Materials and methods

2.1. Wetland

A set of 12 experimental surface flow wetlands were used for determining wetland biomass yield and corresponding nutrient content, and for supplying substrate for mushroom production. The wetlands were built in 2014 in the catchment area of the Albäcken River, Scania, southern Sweden (55°23′N, 13°05′E). Each of the 12 experimental wetlands (40 m^2) is based on a two-stage ditch measuring 4 m × 10 m at the base. Of the width, 2.5 m form a bench, while 1.5 m form a channel with 1:2 bank slope and a depth of 0.4 m. At normal water level, the channel fills up to the bank height, i.e., a water level of 0.4 m. The wetlands are fed with water from a 5000 m^2 large reservoir with a mean water depth of 0.5 m and maximum depth of 1 m receiving nutrient-rich runoff from ~300 ha of agricultural land. In 2014, the wetlands were planted with two-year-old common reed (*Phragmites australis*) plants, at a density of two plants per square meter. After sampling for analysis and mushroom production in 2016, six of the wetlands were harvested completely and the biomass was removed, in order to assess the impact on biomass yield in the following year.

2.2. Mushroom production

2.2.1. Inoculum production

The non-spore producing oyster mushroom strain *Pleurotus ostreatus* M2140 was used in the experiments and was obtained from Mycelia, Belgium. Inoculum was produced by propagating the strain for 12 days on sterile wheat grain amended with 4% $CaCO_3$ and 2% $CaSO_4$ (dry weight/dry weight) at 27 °C.

2.2.2. Substrate production

Wetland biomass, dried as described below, was cut into 2–4 cm pieces and rewetted to a moisture content of 70%. The biomass was then packed into gas-permeable bags suitable for mushroom production (Sac O2, Nevele, Belgium). The bags were autoclaved and inoculum was added in a concentration of 5% (dry weight/dry weight) after the substrate had cooled down. The experiment was performed with three replicates and repeated twice.

2.2.3. Cultivation conditions

The inoculated substrate bags were kept at 24 °C and 75% humidity in a growth chamber. On day 19, the substrate was densely colonized by mycelium and the bags were opened, the temperature was lowered to 12 °C, and humidity was raised to 90%. The temperature was increased to 20 °C on day 22 and the mushrooms were harvested on day 26. Only the first flush was harvested and analyzed.

2.3. Analysis

2.3.1. Wetland biomass yield and composition

In 2016, biomass samples from the experimental wetlands were taken on one harvest occasion, on August 15. At the time of sampling, common reed covered all wetland areas, resulting in 12 replicates. In each wetland, emergent biomass was cut at 15 cm above normal water level in three 1.0 m × 0.5 m plots in the wetland channel and the samples were pooled. In 2017, biomass yield was determined by harvesting one 1.0 m × 0.5 m plot per wetland, on August 28.

The biomass samples were dried at 65 °C for 48 h (until stable weight). The weight before and after drying was used to calculate the moisture content. In 2016, an additional sample of 15 stems per wetland was oven-dried as described above and used for analysis of total nitrogen, total phosphorus, carbon, and potassium content in the biomass, as described below.

2.3.2. Mushroom productivity on wetland biomass

The amount of mushrooms (fresh and dry weight) produced in the first flush was determined. Mushroom production (fresh weight) was related to the amount of substrate (dry matter), in order to determine the biological efficiency (BE) of the substrate, according to the formula:

$$BE = (\text{Amount of mushroom(fresh weight)}/\text{Amount of substrate (dry matter)}) \times 100$$

2.3.3. Chemical analysis

The dried biomass was analyzed for organic carbon and total nitrogen concentration using a Thermo Scientific™ FLASH 2000 CHNS/O Analyzer. For determination of potassium and total phosphorus, the dried biomass was wet-combusted in HNO_3 (65%) using a microwave technique (CEN Mars 5). The concentration of potassium and phosphorus was analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES) at the commercial laboratory Eurofins, Sweden.

The concentrations of cadmium and lead in the wetland biomass were analyzed before and after mushroom production and in the harvested mushrooms. For this analysis, wet combustion was performed as described above and the metals were analyzed by atomic absorption spectrometer (AAS) (Agilent Technologies 200 AA) amended with a

Table 1
Biomass yield of common reed (*Phragmites australis*) in 2016 and 2017 and characteristics of the wetland biomass in 2016. DM = dry matter.

Parameter	Units	Number of samples	Content, mean ± std. dev.	Range
Biomass yield				
2017	[t DM/ha]	12	13.7 ± 6.2	3.3–22.0
2016	[t DM/ha]	12	12.4 ± 3.9	7.5–18.7
Dry matter	[%]	12	41.4 ± 4.2	34.3–48.0
Nitrogen	[mg/g DM]	18	18.9 ± 5.8	13.8–31.5
Phosphorus	[mg/g DM]	18	1.8 ± 0.9	0.1–3.3
Potassium	[mg/g DM]	12	12.8 ± 4.8	1.8–19.7
Carbon	[mg/g DM]	18	423 ± 10	404–440
Carbon/nitrogen ratio	[−]	18	24 ± 5	14–30

graphite tube atomizer (GTA 120). A reference standard (CRM, BCR-679) was also analyzed.

Proximate analysis of ash, crude protein, crude lipid, neutral detergent fiber (NDF), and energy content was performed on the wetland biomass before and after mushroom production. For this analysis, the dried biomass was milled in a coffee grinder (KG40; DeLonghi Appliances, Casula, NSW, Australia) and the ash content was determined by incineration at 550 °C for a minimum of 3 h, and then cooled in a desiccator before weighing. A factor of TN × 6.25 was applied to determine crude protein content (Nordic Committee on Food Analysis, 1976). Crude lipid analysis was performed using an extraction system (Soxtec System HT 1043 Extraction Unit, FOSS Analytical A/S, Hilleröd, Denmark) without acid hydrolysis according to the manufacturer's recommendations (ANKOM Technology, Macedon, NY, USA), with modifications by Hooft et al. (2011). NDF was analyzed according to Mertens (2002) using an amylase neutral detergent method. Energy content was determined with a bomb calorimeter (Parr 6300; Parr Instrument Company, Moline, IL, USA) and expressed as MJ per kg.

2.3.4. Statistics

Statistical analyses were carried out using software package "R" (R Development Core Team, 2011) and Minitab (version 2016). For data handling, MS Excel and the R-package XLSX were used (Dragulescu, 2011). Data were tested for significant differences ($p < 0.05$) using ANOVA and Tukey's post-hoc test and *t*-test.

3. Results and discussion

3.1. Wetland biomass

The harvested wetland biomass consisted exclusively of common reed, with an average biomass yield of 13 ton dry matter per hectare (DM/ha) (Table 1). Moisture content at harvest was 41 ± 4% and 44 ± 4% in 2016 and 2017, respectively. The concentrations of nitrogen, phosphorus and potassium (Table 1) were similar to those reported previously for common reed (Granéli et al., 1982). The resulting removal of plant nutrients from the wetland in 2016 was 234 kg/ha nitrogen, 22.8 kg/ha phosphorus and 158 kg/ha potassium.

Harvesting common reed in six of the 12 wetlands in 2016 did not lead to a significant change in dry matter yield in those wetlands in 2017. Yield of common reed biomass from constructed wetlands harvested in autumn has previously been reported to be around 10 ton DM/ha and year, of which ~25% in the form of leaves (Granéli et al., 1982). In wetlands fertilized with sludge from a wastewater treatment plant, estimated yield of up to 40 ton DM/ha has been reported, while in natural stands an average yield of 5 ton DM/ha has been found for winter harvest (Granéli et al., 1982).

3.2. Mushroom production

Oyster mushroom was able to colonize the wetland biomass and to produce fruiting bodies on this substrate. Similar results were obtained in both experiments and the total time to harvest was 26 days. This time to harvest is well in line with the time needed in commercial production of *P. ostreatus*, which is generally 3–4 weeks (Sánchez, 2010). The harvested fruiting bodies appeared typical for *P. ostreatus*, with heavy, numerous clusters shaded grey-brown (Stamets, 2000). The BE of the substrate was 138 ± 10% which, when expressed as dry weight of mushrooms per unit dry weight substrate, corresponds to 11 ± 1%. The fruiting bodies had a total protein concentration of 18.3 ± 2.8%, based on a conversion factor of 4.38 for total nitrogen concentration (Barros et al., 2008). The BE values and protein content reported previously for *P. ostreatus* vary widely between different substrates. For example, in a study by Koutrotsios et al. (2014), the BE ranged from 20 to 140% and the protein concentration from 3 to 17%. These differences can be explained by the physical and chemical composition of the substrate. The results obtained in the present study demonstrated that reed is well-suited as a substrate for production of *P. ostreatus*.

The results obtained in the present study suggest potential mushroom production of 17.1 t/ha or 1.4 t DM/ha, with a protein yield of 250 kg/ha of harvested wetland, when production is based on reed. However, in order to achieve mushroom production based on wetland biomass on a larger scale, some technical issues need to be addressed. The first of these is harvesting and removal of biomass, which is also important for removal of assimilated plant nutrients (Kadlec and Wallace, 2009). Other technical issues relate directly to mushroom cultivation. An important and energy-demanding step is inactivation of indigenous microorganisms in the harvested biomass. In the present study the biomass was autoclaved for this purpose. In commercial production of oyster mushrooms pasteurization at 60 °C for 1–2 h is commonly applied, and low-tech options for small producers are available (Sánchez, 2010). Materials such as spawn and bags are needed, which can easily be obtained from companies specializing in mushroom production. For successful mushroom production, climate control equipment is crucial in order to stabilize room temperature and humidity. However,

Table 2
Carbon/nitrogen (C/N) ratio and concentration of total nitrogen (TN, mg/g DM), total phosphorus (TP, mg/g DM), cadmium (Cd, μg/kg DM), and lead (Pb, μg/kg DM) in wetland biomass before and after (spent substrate) mushroom production and in the harvested mushrooms. Mean ± standard deviation, n = 3.

	C/N ratio	TN	TP	Cd	Pb
Wetland biomass	29.1 ± 1.1a*	14.1 ± 0.3a	3.1 ± 0.3a	11.7 ± 1.5a	164.8 ± 34.7a
Spent substrate	26.0 ± 1.0b	16.0 ± 0.7b	1.3 ± 0.2b	26.1 ± 4.6b	418.5 ± 64.8b
Mushrooms	10.2 ± 1.6c	41.8 ± 6.4c	10.9 ± 1.6c	45.9 ± 13.2c	44.9 ± 37.8c

* Values within columns followed by different letters are significantly different ($p < 0.05$).

Table 3
Proximate chemical composition (% dry matter, DM) and energy content (MJ/kg DM) of wetland biomass before and after mushroom production (spent substrate). Mean ± standard deviation, n = 3.

	Ash	Crude protein	Neutral detergent fiber	Crude lipid	Energy content
Wetland biomass	10.6 ± 0.4a*	8.8 ± 0.2a	65.1 ± 0.4a	1.2 ± 0.06a	17.6 ± 0.2a
Spent substrate	7.3 ± 0.5b	10.0 ± 0.4b	43.3 ± 6.4b	0.9 ± 0.04b	17.8 ± 0.3a

* Values within columns followed by different letters are significantly different ($p < 0.05$).

low-tech solutions such as air humidifiers are also available for this purpose.

As previously pointed out, the presence of potential contaminants and the risk of transmission of these into the food chain must be considered in any residue-based process. Pollution of the environment by heavy metals due to industrial and agricultural activities is well documented and has occurred world-wide over the past two centuries (Monteiro et al., 2012). The concentration of heavy metals in plant biomass vary depending on the availability of metals in the surrounding soils, which in turn depends on atmospheric deposition, fertilizer use, total metal content, pH, and soil properties (Six and Smolders, 2014). It should also be pointed out that some soils, e.g., alum shale, have a naturally high concentration of heavy metals such as cadmium, which adds to the final soil cadmium concentration (Söderström and Eriksson, 2013).

As mushrooms are well-known for accumulating heavy metals (Huang et al., 2015), cadmium and lead content was determined in both fruiting bodies and substrate in the present study. The concentration of cadmium in the fruiting bodies was 45.9 ± 13.2 μg/kg DM, which was an almost four-fold increase compared with the concentration in wetland biomass before mushroom production. The concentration of lead in the fruiting bodies was 44.9 ± 37.8 μg/kg DM, which was considerably lower than the concentration in wetland biomass before mushroom production (Table 2). Thus, bioaccumulation was confirmed for cadmium, but not for lead. These results are in agreement previous findings of higher bioaccumulation of cadmium compared with lead in mushrooms (Huang et al., 2015; Liu et al., 2015).

Limits on cadmium concentrations in food items have been set by the European Commission, with a general limit of 1.0 mg Cd/kg fresh weight for mushrooms. However, for the most frequently traded mushrooms, including oyster mushrooms, the limit is 0.2 mg Cd/kg fresh weight (EU, 2006). Under the same regulation, the limit for lead in cultivated mushrooms is 0.3 mg/kg fresh weight. The water content in the fruiting bodies obtained in the present study was 92.3 ± 0.5% and the concentration of cadmium and lead in the fruiting bodies expressed as fresh weight (FW) was 3.5 ± 1.0 μg Cd per kg and 3.4 ± 2.6 μg Pb per kg. Thus, despite the bioaccumulation observed for cadmium, the amount of heavy metals observed in the fruiting bodies was considerably below the statutory limit for traded mushrooms.

3.3. Spent substrate

After mushroom harvest, the wetland biomass had lost 47 ± 2% of its initial dry weight and there was a slight but significant increase in total nitrogen concentration in the spent substrate. The total phosphorus concentration, on the other hand, was significantly decreased in the spent substrate. An increase in the content of heavy metals (cadmium and lead) was observed in the spent substrate compared with the initial concentration, due to concentration of the metals by loss of biomass (Table 2).

On comparing the proximate composition of the wetland biomass before and after mushroom production, significant differences were found (Table 3). Concentrations of ash, NDF, and crude lipid were significantly lower and protein significantly higher in the biomass after harvest, while no differences were observed in energy content.

Reed has a history of being used as a fodder plant and is still used for this purpose in some parts of the world (Köbbing et al., 2013). Given the high NDF and moderate crude protein content of wetland biomass after mushroom production, it may have potential for use in ruminant feed, i.e., as roughage or in silage. The mycelium can enrich the substrate with various bioactive compounds that can act as functional additives in feed (Ahmed et al., 2017; Van Doan et al., 2017). Oyster mushroom in particular is known for producing several bioactive compounds with possible pharmaceutical applications (Sánchez, 2010; Reis et al., 2012), which may indicate a direction for future research. In this context, the increased concentrations of cadmium and lead in the spend substrate compared with the initial substrate need to be addressed. This increase is related to the reduction in organic carbon in the initial substrate due to fungal degradation and respiration. However, the amount of heavy metals observed in the spent substrate did not exceed the statutory limit for cadmium and lead content in complete feed, which is set at 0.5 and 5 mg per kg feed with a moisture content of 12%, respectively (EU, 2013).

4. Conclusions

From the results obtained in the present study, it can be concluded that common reed is a suitable substrate for production of oyster mushrooms. No amendment of the biomass was necessary and the harvested mushrooms were of high quality, with morphology typical of *P. ostreatus*, a protein concentration of 18%, and low levels of heavy metals. Thus, the plant nutrients assimilated in wetland biomass, thereby decreasing the risk of eutrophication in receiving water, can also be utilized for direct production of high-quality food. Furthermore, the biomass remaining after mushroom harvest is partly degraded and still maintains a suitable nutrient composition for possible use in ruminant feed. Wetlands thus provide a range of valuable ecosystem services and the concept explored in the present study extends their versatility.

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Conflict of interest

The authors declare that they have no conflict of interest.

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